The potential for endozoochorous dispersal of temperate fen plant species by free-roaming horses

Peter A. Stroh, Francine M. R. Hughes & J. Owen Mountford

Stroh, P.A. (corresponding author, peter.stroh@anglia.ac.uk) & **Hughes, F.M.R.** (francine.hughes@anglia.ac.uk): Animal and Environment Research Group, Department of Life Sciences, Anglia Ruskin University, East Road, Cambridge CB1 1PT

Mountford, J.O. (om@ceh.ac.uk): NERC Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, OX10 8BB, UK

Running head

Dispersal of temperate fen plants by free-roaming horses

Manuscript submission category:

Ordinary paper

Word count

Abstract	332 words
Main text	4160 words
Acknowledgements	72 words
Number of Tables	3
Number of Figures	2

Abstract

Questions: Can free-roaming Konik horses digest and subsequently disperse seeds of plant species growing in a temperate fen environment? Which species have the potential to be dispersed internally (endozoochory), and do they share common plant and seed traits? How could potential dispersal via endozoochory impact upon grazing management and the natural recolonisation of wetland vegetation at a landscape scale?

Location: Wicken Fen National Nature Reserve (NNR), Cambridge, UK.

Materials and Methods: Twelve dung samples were collected for one calendar year (June 2009-May 2010) from a free-roaming herd of Konik horses grazing vegetation at Wicken Fen NNR. Germinable seed content of the dung was determined by means of a seedling emergence study. Species presence and estimated cover/abundance were recorded for the grazed (background) vegetation in spring and summer 2009.

Results: Almost one third of all species present in the background vegetation were also identified in the dung samples. There was an equal split between graminoids and forbs, but substantially more graminoid germinable seeds. Species were dispersed year round, with the number of species peaking in September and again in December. Of the thirteen traits measured, there were significant differences between dung and background vegetation for seven traits. Mean values for seed release heights (minimum and maximum) and dispersule weight were significantly lower in species found in the dung, whilst dung species had significantly greater numbers of seeds per shoot, inferred Ellenberg nitrogen and light values, and seed bank persistence. Explanations for the significantly different species traits in the dung compared with the standing vegetation included adaptation for surviving the digestive process and dispersal strategies. Species traits associated with endozoochorous dispersal shared similarities with the traits necessary for survival in the soil seed bank.

Formatted: Font:

Conclusion: Year-round free-roaming grazing was shown to have the potential to make a considerable contribution to long-range dispersal of species growing in temperate fen habitats, and is likely to complement alternative dispersal strategies. However, germination and establishment of species post-dispersal will be governed by a wide range of environmental conditions.

Keywords

Konik; landscape scale; plant traits; seedling emergence; wetland restoration; Wicken Fen

Introduction

Large herbivores can contribute to species dispersal via the ingestion and subsequent defecation of plant propagules (endozoochory) (Janzen 1984; Vellend et al. 2003). Germinable seed were observed to be retained in deer for more than three days (Vellend et al. 2003), cattle for two to three days (Simao Neto et al. 1987) and horses for up to twelve days (Janzen 1982). Consequently, and depending on the home range of the grazing animal, consumed seeds have the potential to be deposited in dung many kilometres from the parent plant (Pakeman 2001).

Deleted: may

Free-roaming wild herbivores would once have played an important role in the postglacial transportation of species across large distances (Heinken et al. 2002; Mouissie et al. 2004; Jaroszewicz et al. 2009). In more recent times, traditional grazing practices involving the seasonal movement of domesticated livestock (e.g. transhumance; droving) would have facilitated the dispersal of species along defined migration routes (Duffey 1968; Bruun & Fritzbøger 2002). However, there have been substantial changes to or abandonment of traditional grazing systems over much of north-west Europe throughout the twentieth century (Laiolo et al. 2004; Peco et al. 2005; Poschlod, Bakker & Kahmen 2005).

Changes in grazing practices have coincided with the widespread destruction of temperate fen ecosystems (Moore 1997; Jansen 2000), so that fragments of remnant fen habitat are now isolated within a highly modified landscape. The loss of dispersal vectors and metacommunities [regional species pools connected by dispersal mechanisms (Leibold et al. 2004)] reduces the probability of populations colonising or recolonising an area (MacArthur & Wilson 1967; Hanski 1998) and therefore increases the probability of local extinctions (Harrison et al. 2000). The survival chances of species populations is therefore likely to be enhanced for those species that are able to disperse across long distances (Ozinga et al. 2004) and into suitable habitat. The ability to disperse over long distances is also likely to be

advantageous in the face of future climate change. There is, therefore, a pressing need to a) re-establish connectivity between intact nature reserves or to substantially expand reserve boundaries through habitat restoration and b) quantify the long-distance (i.e. >100m following Cain et al. 2000) dispersal potential of plant species to better inform the strategies used in restoration.

In an attempt to repair ecological fragmentation, habitat restoration projects are increasingly taking place over a landscape-scale (e.g. Wigbels 2000; Colston 2004). In south east lowland UK, a number of such projects have been initiated in low-lying areas of fenland that were drained for agriculture in the seventeenth century. These projects aim to increase the area of wetland habitats and the levels of connectivity in the landscape. However, dispersal vectors such as hydrochory (e.g. natural flooding events) which would have been present in these low lying fen areas are now almost absent in the landscape due to increased flood control, and many species classified as wind dispersed (e.g. Kleyer et al. 2008) have been shown to rarely travel >100m from the parent plant (Bullock & Clarke 2000; Jongejans & Telenius 2001; Tackenberg et al. 2003). Consequently, the contribution of large herbivorous mammals to the long range dispersal of fen vegetation may be of considerable importance to the developing habitats in the new landscape-scale restoration projects.

Plant functional traits associated with successful internal dispersal of diaspores are hypothesised to be linked with edible seed casings, seed weight, seed shape and resistance to digestive processes (e.g. Janzen 1984; Pakeman et al. 2002; Couvreur et al. 2005). Numerous studies have examined the potential for endozoochorous seed dispersal across a range of dry grassland and heathland habitats (Welch 1985; Middleton & Mason 1992; Malo & Suárez 1995; Miller 1996; Eichberg et al. 2007; Kuiters & Huiskes 2010). However, there are very few studies concentrating on endozoochorous transport within wetland systems (Pakeman et al. 2002), and none within a temperate fen environment. In order to learn more about the

potential for livestock to carry out long-range dispersal of plants associated with such habitats, the main objectives of the study described here were to:

1. Determine the viable seed content of dung collected from free-roaming Koniks grazing a species-rich UK fenland National Nature Reserve (NNR)
2. Examine temporal variation in dung species composition
3. Investigate the similarities and differences between species transported by endozoochory and background vegetation traits
4. Discuss the implications for grazing management and landscape-scale restoration

Methods

Study site

Wicken Fen National Nature Reserve (NNR), situated 25 kilometres north of Cambridge (UK) (52°18'24N, 0°16'51E), is one of the last undrained remnants of what would once have been a vast interconnected landscape of floodplain mire in lowland East Anglia some 3,850km^2 in size (Moore 1997). The NNR is 159 hectares (ha) in size and contains plant assemblages associated with low fertility floodplain fens on undrained alkaline peat (McCartney & de la Hera 2004), including fen meadow, sallow carr and tall-herb fen. The study site is located within a 56.2ha unfenced section of the reserve known as 'Verralls Fen', which is bounded on all sides by water-filled ditches. Following substantial scrub clearance operations from 2000-2001, the area has been extensively grazed year-round by a free-roaming herd of Polish Konik ponies (henceforth referred to as Koniks, as the Polish word *konik* translates as "horse" or "pony"). Koniks were introduced because of similarities to the original Eurasian wild horse, including a resilient immune system, tolerance for harsh and wet conditions and an ability to graze coarse vegetation.

Deleted: horses

Wicken Fen Vision

The Wicken Fen Vision is a landscape-scale restoration project bordering the NNR. The project is in the process of acquiring c.3000 ha of land to begin to address the problems of species isolation and dispersal. Restoration of vegetation within the project areas is based on an 'open ended' approach (Hughes et al. 2011) which promotes natural colonisation by flora and fauna and the repair (*sensu* Whisenant 1999) of ecosystem structure and biodiversity to establish ecosystem resilience (Breedlow et al. 1988), rather than seeking to replicate historic reference systems. The restoration areas are also grazed by Koniks but are not currently linked to the NNR. In anticipation that this link may one day be made, this study investigated the seeds in the dung of the horses grazing Verralls Fen on the NNR to explore the potential for these horses to transfer species from the NNR to the restoration land.

Dung sampling

Dung from the Konik herd (which numbered 13 animals in total) was collected on seven separate occasions (once every three weeks) throughout the main plant fruiting season between the months of June and October 2009. On each visit, samples were collected from the first three Koniks to defecate. Five additional visits to collect horse dung were undertaken at regular intervals outside of the main fruiting season so that an analysis for a full calendar year could be undertaken. To avoid contamination by wind-blown seed, only dung which was observed as freshly deposited was collected. The lowermost layer was left behind to avoid contamination by seed lying on the soil surface. Fresh dung collected at each visit was homogenised and immediately placed in cold storage (constant 3^{o}C) for four weeks to promote stratification. Samples were then spread thinly and fully dried in a sealed room at a constant 30^{o}C for 5 to 7 days. Once dried, a sub-sample of 1kg (dry weight) representing a single site visit (hereafter referred to as a 'sample') was stored at -20^{o}C to maintain seed viability (following Linington 2003) until commencement of the seedling emergence experiment in April 2010.

175 *Germinable seed content*

176 In April 2010, all samples (n=12) were transferred to a glasshouse located within Cambridge
177 University Botanic Gardens. Before placing the material into seed trays, each sample was
178 separated into four sub-samples of 250g (dry weight) and homogenised in a volume of
179 distilled water (following Kuiters & Huiskes 2010). The resultant 'slurry' was then spread in
180 a thin layer onto a mixture of sand and sterilised peat (1:1). Trays without dung samples
181 were also present to detect germination from the compost and dispersal into the greenhouse.
182 This method is similar to the one devised by ter Heerdt et al. (1996), but does not employ the
183 use of fine sieves due to the high content of dead vegetative matter present in the dung.
184 Preparation of dry dung by grinding was not used as this may damage large seeds (Cosyns &
185 Hoffmann 2005a).

186 Seed trays (n=48, excluding controls) were placed on capillary matting and regularly
187 watered from below. Seedlings were identified at the earliest stage possible, recorded and
188 then removed. Seedling emergence was recorded for six months. Species that we were not
189 able to identify at seedling stage were potted on and identified when diagnostic features
190 became visible.

191 *Background vegetation*

192 A comprehensive list of all species (following Stace 2010) present in the vegetation
193 (hereafter referred to as the background vegetation) was recorded in spring and summer 2009.
194 Cover/abundance for the entirety of the study area was estimated for each background species
195 through the undertaking of a series of line-transect walks within the study area.
196 Cover/abundance was estimated using the DOMIN scale (1 = <4% (few individuals); 2 =
197 <4% (several individuals); 3 =<4% (many individuals); 4 = 4–10%; 5 = 11–25%; 6 = 26–
198 33%; 7 = 34–50%; 8 = 51–75%; 9 = 76–90%; 10 = 91–100%). However, the size of the
199 grazed area (56ha) within which horses were free to graze and the subsequent uncertainty

surrounding where and when species had been consumed meant that a strict quantitative comparison of species occurring in the background vegetation and those germinating from the dung was not possible**.** Species presence/absence data were therefore used to compare functional traits of species that were found in the dung with traits of species that were in the background vegetation

Data analysis

Species functional traits were chosen to reflect both seed characteristics found to be important in endozoochory in the literature and plant characteristics that influence choice of species grazed by horses. Information on species functional traits was gathered from three principal sources. Inferred Ellenberg values for nitrogen (N), light (L) and moisture (F) were taken from PLANTATT (Hill et al. 2004). Dispersule weight, shape, and Competitor-Stress tolerator-Ruderal (C-S-R *sensu* Grime 1977) characteristics was taken from the Electronic Comparative Plant Ecology (Grime, Hodgson & Hunt 2007). Information on seed bank longevity, seed release heights and number of seed produced per shoot was assembled from the LEDA traitbase (Kleyer et al. 2008). Vegetation classification (following Rodwell 1991) for dung species content was carried out using the programme MAVIS [Modular Analysis of Vegetation Information Systems] (Smart 2000).

Following the amalgamation of seed tray data for each sample, mean trait values for each dung sample (n=12) were calculated by presence of species in each sample and the trait score. Mean trait values for background vegetation were calculated by presence of all recorded species and the trait score. One-sample t-tests were performed to test for differences in dung and background vegetation traits. For each trait, the mean for each sample (n=12) was used to test against a hypothesised mean. For the purpose of this analysis, the background vegetation mean replaced zero as the hypothesised mean. Statistical analyses were carried

Comment [mh1]: Compare non-germinating background vegetation species with germinating background vegetation species

224 out using Minitab v.14. Abundance data from the seedling emergence study was used to
225 examine temporal patterns in dung species content.

226 **Results**

227 The background vegetation in the grazed study area comprised 135 species recorded across a
228 complex mosaic of four main vegetation types; *Peucedano-Phragmitetum australis* tall-herb
229 fen, *Cirsio-Molinietum* fen meadow, *Phragmitetum australis* reed bed and *Cladietum marisci*
230 sedge-beds (Table 1). Background species with an estimated cover/abundance of ≥10%
231 across the 56ha site included graminoids such as *Juncus subnodulosus*, *Calamagrostis*
232 *epigejos*, *Calamagrostis canescens*, *Cladium mariscus*, *Phragmites australis*, *Phalaris*
233 *arundinacea*, *Poa trivialis* and *Molinea caerulea*. Many graminoids and forbs which were
234 recorded at the study site were locally abundant within areas of ≤1ha, but did not comprise
235 ≥10% cover/abundance when the study area was considered as an entire unit. Two forbs
236 (*Thyselium palustre* and *Lathyrus palustris*) categorised by Cheffings & Farrell (2005) as
237 'vulnerable' and 'near threatened' respectively in a UK context were recorded in low
238 numbers within the background vegetation but were not recorded from the dung samples. A
239 third species, *Viola persicifolia* (classified as 'critically endangered'), is known to have
240 occurred at the study site until recently but was not recorded in the background vegetation or
241 the dung samples in the current survey.

242 All species which germinated from the seedling emergence experiment were also found
243 within the background vegetation. A total of 2548 seedlings of 41 species (30.37% of all
244 background species) from 18 plant families were recorded from the dung samples (Table 2).
245 Graminoids accounted for 48.78% of all species and 82.03% of all seedlings, whilst forbs
246 made up 51.22% of species but only 17.97% of all seedlings. The mean number of
247 germinable seeds for each collection date was 212.33/kg, with the highest number of seeds/kg
248 found in mid-August (720/kg) (figure 1). The greatest number of species was found in the

Comment [mh2]: Combine table 1 and 2; this will more clearly show the non-germinating background vegetation species

Comment [mh3]: Nonetheless *Plantago major, Poa annua* and *P. pratensis* are mentioned to germinate from the dung (table 2) and are not mentioned from the background vegetation (table 1).

Comment [mh4]: Combine with table 1

mid-September sample, and the lowest number in the mid-June sample (figure 2). The *Juncaceae* and *Poaceae* were the most dominant plant families in the dung samples, accounting for 34.14% of all species (12.19% and 21.95% respectively), and 80.49% of all seedlings (64.83% and 15.66% respectively). *Berula erecta*, *Lythrum salicaria* and *Phragmites australis* were only present in winter samples.

A small number of species that germinated from the samples could be categorised as specialist fen plants within a UK context (Rodwell 1991) (e.g. *Berula erecta*, *Juncus subnodulosus*, *Carex panicea*, *Galium uliginosum*), whilst the majority of germinants were generalists known from a variety of other UK grassland or woodland habitats (e.g. *Calamagrostis epigejos*, *Carex flacca*, *Carex otrubae*, *Plantago lanceolata*, *Urtica dioica*). However, a vegetation classification using all species present in the dung resulted in close affinities to a *Cirsio-Molinietum* fen meadow assemblage.

Deleted: *Juncus-*

Deleted: *um*

Traits for background vegetation and dung samples are summarised in Table 3. There were significant (P=≤0.05) differences between dung and background vegetation for seven of the thirteen traits measured. Mean values for seed release heights (minimum and maximum) and dispersule weight were significantly lower in species found in the dung, whilst dung species had significantly higher seed bank persistence, inferred Ellenberg nitrogen and light values, and marginally significantly greater numbers of seeds per shoot.

Background species	DOMIN score	Background species	DOMIN score
*Agrostis stolonifera**	5	*Lycopus europaeus**	2
Angelica sylvestris	1	*Lysimachia vulgaris*	2
*Berula erecta**	2	*Lythrum salicaria**	2
Calamagrostis canescens	5	*Mentha aquatica**	3
*Calamagrostis epigejos**	4	*Molinia caerulea*	4
Calystegia sepium	3	*Myosotis laxa*	1

*Carex disticha**	2	*Phalaris arundinacea*	4
*Carex flacca**	1	*Phleum pratense s.s.**	2
Carex hostiana	2	*Phragmites australis*	4
Carex lepidocarpa	2	*Poa trivialis**	4
*Carex otrubae**	3	*Polygala vulgaris*	1
*Carex panicea**	2	*Populus canadensis**	1
Carex riparia	1	*Potentilla erecta*	1
Centaurea nigra	1	*Potentilla reptans**	2
Cirsium dissectum	2	*Primula veris*	1
Cirsium palustre	2	*Prunella vulgaris**	2
Cladium mariscus	4	*Ranunculus flammula*	2
Dactylhoriza incarnata	1	*Rhinanthus minor*	3
Deschampsia cespitosa	2	*Salix cineraea**	3
*Epilobium cilliatum**	1	*Salix repens*	3
*Epilobium hirsutum**	3	*Samolus valerandi**	2
*Epilobium tetragonum**	1	*Schrophularia auriculata*	2
*Eupatorium cannabinum**	3	*Scuttelaria galericulata*	3
Fillipendula ulmaria	2	*Silene flos-cuculi*	2
Galium palustre	3	*Solanum dulcamara**	2
*Galium uliginosum**	2	*Succisa pratensis*	2
Glechoma hederacea	3	*Symphytum officinale*	3
*Glyceria declinata**	1	*Thalictrum flavum*	2
*Glyceria fluitans**	1	*Thelypteris palustris*	2
Hydrocotyle vulgaris	2	*Thyselium palustre*	1
Iris pseudacorus	3	*Trifolium pratense**	1
*Juncus articulatus**	2	*Triglochin palustre*	1
*Juncus bufonius**	2	*Typha latifolia**	2
*Juncus effusus**	1	*Urtica dioica**	2
*Juncus inflexus**	3	*Valeriana dioica*	2
*Juncus subnodulosus**	4	*Valeriana officinalis*	2

Deleted: *Rhianthus*

Deleted: *Trifoilum*

Lathyrus palustris	1	*Veronica beccabunga**	2
Linum catharticum	2	*Veronica catenata**	1
*Luzula multiflora**	1	*Viccia cracca*	3

Table 1. Plant species recorded from the background vegetation.

Species which were estimated at ≥4% cover across the 56ha study area are listed, together with species estimated as ≤4% cover but categorised as constituent species of a main fen vegetation type (Rodwell 1992) and/or species which germinated from the dung samples. Species marked with an * denote presence in both the background vegetation and dung samples. DOMIN scale: 1 = <4% (few individuals); 2 = <4% (several individuals); 3 =<4% (many individuals); 4 = 4–10%; 5 = 11–25%. Cover/abundance for an individual species did not exceed 25%.

species	Family	Mean seed abundance/kg	presence (no. of samples)	peak abundance (month)
Agrostis stolonifera	Poaceae	27.64	11	August
Berula erecta	Apiaceae	12	2	April
Calamagrostis epigejos	Poaceae	1.67	3	September
Carex disticha	Cyperaceae	3.67	3	August
Carex flacca	Cyperaceae	4.67	3	July
Carex otrubae	Cyperaceae	3.33	3	July
Carex panicea	Cyperaceae	2.5	4	August
Epilobium ciliatum	Onagraceae	2.5	2	August
Epilobium hirsutum	Onagraceae	4	4	October
Epilobium tetragonum	Onagraceae	1	2	December
Eupatorium cannabinum	Asteraceae	1.75	4	October
Galium uliginosum	Rubiaceae	2.33	3	January

Glyceria declinata	Poaceae	3	1	December
Glyceria fluitans	Poaceae	2	2	December
Juncus articulatus	Juncaceae	97.33	9	August
Juncus bufonius	Juncaceae	63.63	8	August
Juncus effusus	Juncaceae	2.5	2	September
Juncus inflexus	Juncaceae	18.89	9	August
Juncus subnodulosus	Juncaceae	10.88	8	September
Luzula multiflora	Juncaceae	2	1	September
Lycopus europaeus	Lamiaceae	4.5	2	January
Lythrum salicaria	Lythraceae	3	1	December
Mentha aquatica	Lamiaceae	2.67	3	October
Phleum pratense	Poaceae	1	1	October
Phragmites australis	Poaceae	5	1	December
Plantago lanceolata	Plantaginaceae	2.5	2	August
Plantago major	Plantaginaceae	14.14	7	August
Poa annua	Poaceae	3.33	3	July
Poa pratensis	Poaceae	2.25	4	August
Poa trivialis	Poaceae	11.6	5	August
Populus canadensis	Salicaceae	1.5	2	July
Potentilla reptans	Rosaceae	4	1	September
Prunella vulgaris	Lamiaceae	2.57	7	September
Salix cinerea	Salicaceae	1.5	2	July

Samolus valerandi	Primulaceae	5.17	6	August
Solanum dulcamara	Solanaceae	5	2	October
Trifolium pratense	Fabaceae	2	2	December
Typha latifolia	Typhaceae	3	1	July
Urtica dioica	Urticaceae	45.5	4	September
Veronica beccabunga	Scrophulariaceae	6	2	September
Veronica catenata	Scrophulariaceae	4.33	3	July

277

278 Table 2. Plant species which germinated from Konik dung samples. Mean abundance is measured as seeds/kg
279 dry dung. Presence refers to the total number of samples (n=12) a species was recorded from. Peak abundance
280 refers to the sample month with the highest recorded number of seedlings per species.

281

Trait	Background vegetation (mean)	Konik dung (mean)	P value	t
Dispersule weight (mg)	2.88	2.08	<0.001	-11.61
Dispersule shape (length/breadth ratio)	1.78	1.8	0.668	0.44
Ellenberg Light (L)	6.95	7.05	0.038	2.36
Ellenberg Nitrogen (N)	5.07	5.48	0.046	1.96
Ellenberg Moisture (F)	6.93	7.38	0.124	1.67
Competitor value (C)	0.43	0.45	0.61	0.52
Stress-tolerator value (S)	0.25	0.24	0.348	-0.98
Ruderal value (R)	0.33	0.31	0.698	0.4
Seed bank longevity	0.33	0.48	<0.001	8.61
seed release height (cm) min	32.75	26.49	<0.001	-5.02
seed release height (cm) max	111.62	86.31	<0.001	-8.61
seed number per shoot	15497.8	32420.55	0.052	2.18

Table 3: Results of analysis of plant traits (overall mean values) for species recorded in the standing vegetation and in the Konik dung using one-sample t-tests. Mean values for each trait for species germinating from dung samples were calculated by presence of species in each sample (n=12) and the associated trait value. Background vegetation trait values were calculated by the presence of species in the vegetation and the associated trait value. Dispersule weight: 1 = ≤20 mg; 2 = 0.21-0.50 mg; 3 = 0.51-1.00 mg; 4 = 1.01-2.00 mg; 5 = 2.01-10 mg. Dispersule shape is based on length/breadth ratio, where 1= ≤1.5; 2 = 1.5-2.5; 3 = ≥2.5. Ellenberg L (light): 5 = plants of semi-shade, rarely in full light; 6 = intermediate between 5 and 7; 7 = plants of generally well lit places. Ellenberg N (nutrient): 3= infertile sites; 4 = intermediate between 3 and 5; 5 = sites of intermediate fertility; 6 = intermediate between 5 and 7; 7 = rich, fertile soils. Ellenberg F (moisture): 5 = average dampness; 6 = intermediate between 5 and 7; 7 = constantly moist or damp soils. Seed bank longevity index ranges from short-lived (0) to long-lived (1).

Comment [mh5]: From the text, I conclude that the plant traits values of **all** background vegetation species are included in the calculation of a mean, and not merely the species that did not germinate from the dung samples; nonetheless the purpose of this table and comparison is to find out whether these plant traits are significantly different between germinating seeds and non-germinating seeds that are present in the background vegetation. You would probably find much stronger differences between both then in the present case, in which you include all background vegetation species; you should at least add a column with plant trait means of the background vegetation species that did not germinate from the dung samples. Of course, this assumes that seeds of all background vegetation species **could** (not so if the species remain sterile) **and were** digested by the horses (not so if they are toxic or in any other way defended against grazing).

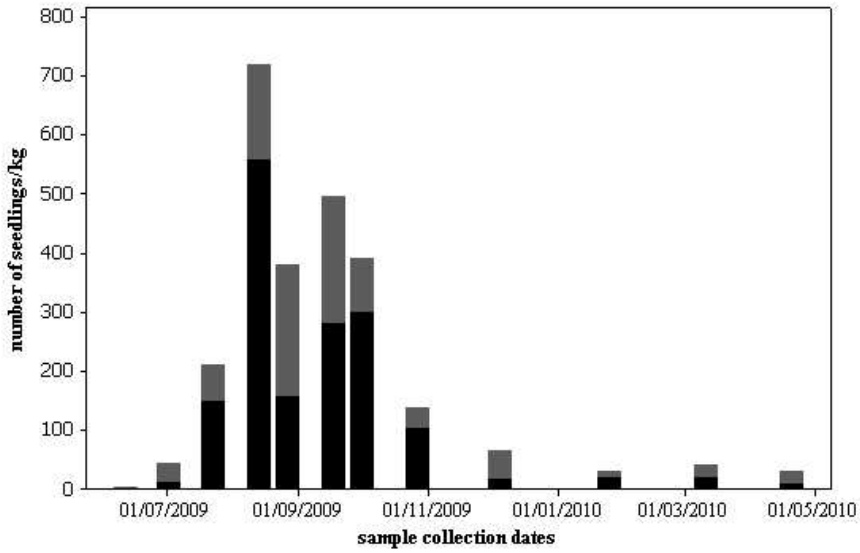

Figure 1: mean number of germinable seeds/kg (dry weight) from dung samples collected June 2009 – May 2010. Black shaded areas represent the number of seedlings belonging to the *Juncaceae*.

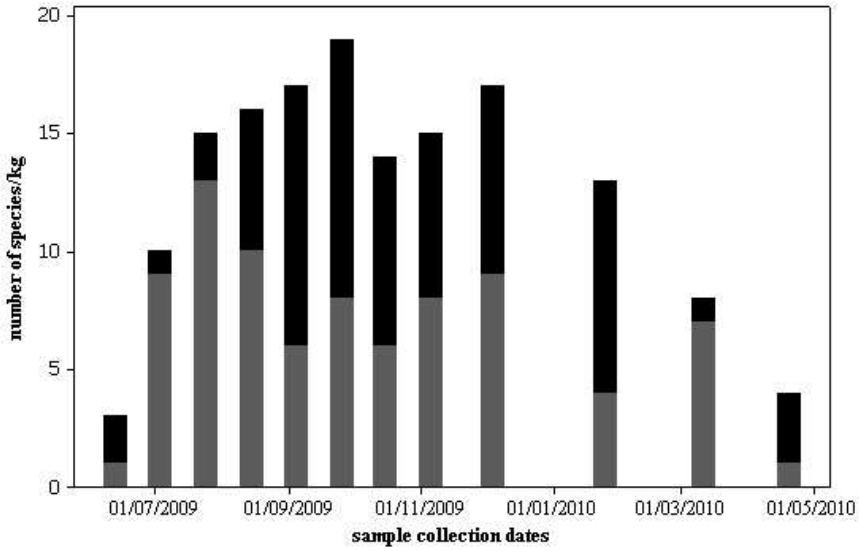

Figure 2: total number of plant species per collection date/dry kg of Konik dung. Black shaded area represents the total number of species which germinated from each dung samples. Grey shaded area represents the number of graminoid species which germinated from each sample.

Discussion

Almost one third of the available species pool was present in Konik dung samples, a value which is similar to that found in previous studies in other habitats (e.g. Malo & Suárez 1995; Traba et al. 2003; Kuiters & Huiskes 2010) and demonstrates the potential importance of endozoochorous dispersal for plants found in temperate fens. It is possible that sub-optimal germination conditions for some species in the seedling emergence study or species rarity in the background vegetation led to an underestimation of plants capable of surviving internal transportation.

Based on our seedling emergence results free-roaming horses have the ability to transport hundreds of thousands of seeds in each calendar year (see also Cosyns et al 2005a; Jaroszewicz et al. 2009; Kuiters & Huiskes 2010) and have the potential to disperse the

Comment [mh6]: The first to mention the enormous potential of endozoochorous dispersal through two horse breeds, among which Konik was Cosyns et al 2005a: table 4.

component species of a range of vegetation types recorded within the study area. The high proportion of viable graminoid seeds recorded from the samples reflects similar results from other endozoochorous studies in other habitats (Welch 1985; Dai 2000; Mouissie 2004; but see Malo & Suarez 1995). However, germination and establishment of internally dispersed seeds under natural field conditions, post-dispersal, is likely to be substantially lower than seedling emergence results suggest as they are affected by a number of biotic and abiotic filters. These include seed predation, modified hydrology or soils, a deficiency of species-specific germination cues and competition from existing species in the sward, (Pywell et al. 2002; Hobbs & Norton 2004) though competition may initially be moderated by the suppression of vegetation resulting from deposition of seeds in a dung package. An example of the disparity between controlled seedling emergence results and natural outcomes is given in a study undertaken by Cosyns et al. (2006) in which less than 5% of the high numbers of viable monocotyledon seeds recorded in seedling emergence studies successfully established in the field after a three month period. In contrast, although their study found that low numbers of dicotyledon seeds were dispersed, they achieved a higher sward cover and contributed to greater species-richness in natural conditions after one year. Consequently, caution should be used regarding the interpretation of results due to considerable differences between glasshouse and field conditions (Eichberg et al. 2007).

The highest number of species per sample occurred in late September, coinciding with the conclusion of flowering and peak seed production for the majority of species within the background vegetation (Stace 2010). The relationship between seed production and dung seed density has previously been demonstrated in studies investigating endozoochorous dispersal in calcareous grasslands (Bakker & Olff 2003) and heathland (Mouissie et al. 2005). Following this peak, the number of species recorded in the dung falls away, before briefly rising to a second peak in December. The fall in the number of species recorded between

these two peaks may be due to seed mortality or to seed fall from species that retain fruits on the parent plant for only a short period of time, combined with a temporary increase in the abundance of palatable species with seed traits that are not conducive to endozoochorous dispersal. The second species abundance peak which then follows in the winter could be linked to a reduced palatable vegetation resource and the browsing of species which retain seeds intact on the parent plant for a prolonged period of time. The presence of relatively high numbers of species in the December sampling date points to the importance of collecting samples outside of the main growing season, particularly as three of the species which were only recorded in the winter months of our study are strongly associated with wetland or fen vegetation. Mouissie et al. (2005) also found that two constituent species of heathland vegetation (*Calluna vulgaris* and *Erica tetralix*) were only dispersed in the winter months.

Plant trait results demonstrated that there was a wide range of characteristics that are conducive to internal dispersal, supporting Janzen's (1984) central hypothesis. Species of fen vegetation dispersed via endozoochory had a more persistent seed bank than species which were not dispersed, were more light-demanding, had higher seed production rates and may also establish in sites of higher fertility. These traits could be viewed as advantageous for dispersal through dung, considering the high level of nutrients contained within the growing medium, the subsequent creation of areas of bare ground and initial low levels of competition from the surrounding vegetation. For example, producing large numbers of small seeds would increase opportunities for consumption by unselective grazing, and the recruitment opportunities created by grazing disturbance would favour species that rely on an open sward and minimal competition for germination and establishment.

Deleted: were

Pakeman et al. (2001, 2002) suggested that the traits which are necessary to survive ingestion by animals are similar to those that permit long term survival in the soil. Our data, together with previous seed bank work undertaken at the study site (Stroh et al. 2010),

support this supposition. It may be that similarities between dispersal strategies reflect traits which are necessary to survive being interred within another medium (e.g. gut, dung or soil) for a prolonged period of time. In contrast, alternative 'external' dispersal mechanisms (ectozoochory) by grazing animals are more likely to transport short-lived seeds and in doing so complement species dispersed by endozoochory (Couvreur et al. 2004).

The two background species categorised as 'rare' in a UK context (*Lathyrus palustris* and *Thyselium palustre*) were not identified in the dung samples. One explanation for this absence may be simply that the seeds of these species were not consumed due to the large size of the study area and/or the low numbers of individual plants in the background vegetation. Alternatively, it may be that the seeds of these species were consumed but did not survive the digestive process due to associated functional traits. As stated earlier, one explanation for the inability to survive the digestive process may be linked to the length of time a seed can survive as a viable germinant in the soil seed bank. Both species are known to have transient (<1 year survival) seed banks (Thompson et al. 1997), and this deterrent to internal dispersal may also be applicable to other temperate fen species (e.g. *Cirsium dissectum*, *Carex lepidocarpa*, *Cladium mariscus*, *Succisa pratensis*, *Rhinanthus minor*) found in the current study which are known to have a transient seed bank too (e.g. Thompson et al. 1997; Matus et al. 2003). In the cases of *L. palustris* and *T. palustre*, long-distance dispersal strategies are more likely to be adapted to hydrochorous dispersal (see Vaughan 1978; Meredith & Grubb 1993).

Deleted: *Rhianthus*

Seed weight was significantly lower for dung-dispersed species, but seed shape was not significantly different from non-dispersed species, with the mean dimensions suggesting a shape which was more elongate than rounded. This was somewhat surprising, as many studies have concluded that small, rounded seeds are adapted to endozoochorous dispersal (e.g. Bruun & Fritzbøger 2002; Pakeman et al. 2002; but see Cosyns & Hoffmann 2005b).

Comment [mh7]: You did not test this, since you included all background vegetation species, also those that did germinate from dung; if you did not, then this should be clearly stated (see also remark on table 3 and line 221

However, the mean seed weight for dispersed species does describe a small and very light seed. Such an inconspicuous size is an advantage during the digestive process as large and heavy diaspores are more likely to be damaged by the molar mill (Murphy et al. 1989). Species that produce small and light seeds are also more likely to produce greater quantities of seed (Jakobsson & Eriksson 2000) and in doing so increase the probability of dispersal potential from the parent plant (Bruun & Poschlod 2006). The results of our analysis with regard to the marginally but still significantly greater number of seeds produced per shoot for sample species support this assertion.

The significant difference between dung samples and background vegetation found for seed release heights may be explained by at least two factors. Firstly, the lower minimum and maximum seed release heights for endozoochorous species are likely to be linked to the grazing strategies of large herbivores, and specifically in this instance Koniks, which preferentially graze a shorter sward because of the higher nutritional value of young plant growth (Van Braeckel & Van Looy 2002 but see Naujeck & Hill 2005). This in turn would inadvertently result in the unselective ingestion of plants and seeds at the same height in the surrounding vegetation as well as those selected for consumption. Secondly, seeds of plants that have a relatively tall mean height in the sward may be more likely to have an anemochorous dispersal strategy, as the release height is such that seeds of tall plants will have a higher probability of clearing the surrounding vegetation (e.g. Davies & Sheley 2007).

Historically (from around 2,500 years ago when the most recent fen peats were formed in East Anglia (Godwin 1939) until fen drainage during the 17th century), wild herbivores would have browsed floodplain fens, but their abundance and hence impact is likely to have been greatest at the floodplain margins where there would have been firmer ground for resting and, for some species, creation of latrines. This combination of increased grazing intensity and nutrient return through dung could have resulted in the development of vegetation

Comment [mh8]:

Comment [mh9R8]: On the long run, grazing does diminish the number of generative shoots, and hence the number of seeds produced.

assemblages containing many species that are now considered typical of “fen meadows”. As human beings began to exploit the wetlands, their impact too would have been greatest at the floodplain margin, creating mowing meadows and herding their livestock on the shallower peat. In situations such as those found at the study site, where NNR vegetation is adjacent to large-scale habitat restoration land, re-instating free-roaming herds which are able to roam from NNR vegetation onto ex-arable restoration land could re-establish previously lost dispersal links through the landscape. Free-roaming grazing animals thus have the potential to be an effective tool for diversifying restoration vegetation, particularly in areas which share similarities to floodplain margins such as wetland drawdown zones.

Conclusions

The loss of landscape dispersal mechanisms has been directly associated with the loss of plant diversity in northwest Europe in the past hundred years (Ozinga et al. 2009). In this context, free-roaming grazing animals used as mobile links between plant populations have the potential to become important vectors in helping to maintain or restore vegetation through natural colonisation and increased gene flow between extant populations. This study has demonstrated that Koniks may disperse the viable seeds of a wide range of temperate fen species throughout the year, and indicates that optimum times for the movement of grazing animals and potential dispersal of germinable seeds are not restricted simply to the growing season. The conservation grazing system at Wicken Fen NNR and the Wicken Vision both have year-round grazing systems with free-roaming Koniks. If a link is created between the NNR and the restoration land, then free-roaming grazing herds have the potential to be an effective tool for diversifying restoration vegetation that, at the Wicken Vision site, is developing through natural regeneration.

Acknowledgements

This research was funded by the Esmée Fairbairn Foundation Grant no. EN 06/2151. We would like to thank staff at Cambridge University Botanical Gardens, National Trust staff at Wicken Fen, and Professor Tim Sparks for valuable help and advice. Two anonymous referees added greatly to the quality of the manuscript. This work forms part of a doctoral study being undertaken by P.A.S. at the Department of Life Sciences, Anglia Ruskin University, Cambridge.

References

Breedlow, P.A., Voris, P. V. & Rogers, L. E. 1988. Theoretical perspective on ecosystem disturbance and recovery. In Rickard, W. H., Rogers, L. E., Vaughan, B. E. & Liebetrau, S. F. (eds.) *Shrub-steppe: Balance and change in a semi-arid terrestrial ecosystem*. (Eds pp 257-269. Elsevier, New York.

Bruun, H.H. & Fritzbøger, B. 2002. The past impact of livestock husbandry on dispersal of plant seeds in the landscape of Denmark. *Ambio* 31: 425–431.

Bruun, H.H. & Poschlod, P. 2006. Why are small seeds dispersed through animal guts: large numbers or seed size per se? *OIKOS* 113: 402-411.

Bullock, J. M. & Clarke, R. T. 2000. Long distance seed dispersal by wind: Measuring and modelling the tail of the curve. *Oecologia* 124: 506–521.

Cain, M. L., Milligan, B. G. & Strand, A. E. 2000. Long-distance seed dispersal in plant populations. *American Journal of Botany* 87: 1217-1227.

Cheffings, C. M., Farrell, L., (Eds), Dines, T. D., Jones, R. A., Leach, S. J., McKean, D. R., Pearman, D. A., Preston, C. D., Rumsey, F. J. & Taylor, I. 2005. *The Vascular Plant Red Data List for Great Britain.* Report for the Joint Nature Conservation Committee, Peterborough, UK.

Formatted: German (Germany)

Colston, A. 2004. Wicken Fen – realising the Vision. *Ecos – British Association of Nature Conservationists* 25: 42-45.

Cosyns, E., Claerbout, S., Lamoot, I. & Hoffmann, M. 2005a. Endozoochorous seed dispersal by cattle and horse in a spatially heterogeneous landscape. *Plant Ecology* 178: 149–162.

Cosyns, E. & Hoffmann, M. 2005b. Horse dung germinable seed content in relation to plant species abundance, diet composition and seed characteristics. *Basic & Applied Ecology* 6: 11-24.

Cosyns, E., Bossuyt, B., Hoffmann, M., Vervaet, H. & Lens, L. 2006. Seedling establishment after endozoochory in disturbed and undisturbed grasslands. *Basic and Applied Ecology* 7: 360-369.

Couvreur, M., Christiaen, B., Verheyen, K. & Hermy, M. 2004. Large herbivores as mobile links between isolated nature reserves through adhesive seed dispersal. *Applied Vegetation Science* 7: 229-236.

Couvreur, M., Cosyns, E., Hermy, M. & Hoffmann, M. 2005. Complementarity of epi- and endozoochory by free ranging donkeys. *Ecography* 28: 37–48.

Dai, X. 2000. Impact of cattle dung deposition on the distribution pattern of plant species in an alvar limestone grassland. *Journal of Vegetation Science* 11: 715–724.

Davies, K.W. & Sheley, R.L. 2007. Influence of Neighboring Vegetation Height on Seed Dispersal: Implications for Invasive Plant Management. *Weed Science* 55: 626–630.

Duffey, E. 1968. Ecological Studies on the Large Copper Butterfly *Lycaena dispar* Haw. *Batavus* Obth. at Woodwalton Fen National Nature Reserve, Huntingdonshire. *Journal of Applied Ecology*5: 69-96.

Eichberg, C., Storm, C. & Schwabe, A. 2007. Endozoochorous dispersal, seedling emergence and fruiting success in disturbed and undisturbed successional stages of sheep-grazed inland sand ecosystems. *Flora* 202: 3–26.

Formatted: French (France)

Formatted: German (Germany)

490 Gardener, C. J., Mcivor, J. G. & Jansen, A. 1993. Survival of seeds of tropical grassland
491 species subjected to bovine digestion. *Journal of Applied Ecology* 30: 75–85.
492 Godwin, H. 1939. Studies in the post-glacial history of British vegetation. II. Fenland pollen
493 diagrams. *Philosophical Transactions of the Royal Society, Ser. B* 230: 239-284.
494 Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its
495 relevance to ecological and evolutionary theory. *The American Naturalist* 111: 1169-
496 1194.
497 Grime, J. P., Hodgson, J. G. & Hunt, R. 2007. Comparative Plant Ecology: A Functional
498 Approach to common British Species. Castlepoint Press; 2nd Revised edition
499 Hanski, I. 1998. Metapopulation dynamics. *Nature* 396: 41–49.
500 Harrison, S., Marson, J. & Huxel, G. 2000. Regional turnover and fluctuation in populations
501 of five plants confined to serpentine seeps. *Conservation Biology* 14: 769–779.
502 Heinken, T., Hanspach, H., Raudnitschka, D. & Schaumann, F. 2002. Dispersal of vascular
503 plants by four species of wild mammals in a deciduous forest in NE Germany.
504 *Phytocoenologia* 32: 627–643.
505 Hill, M. O., Preston, C. D. & Roy, D. B. 2004. *PLANTATT. Attributes of British and Irish*
506 *Plants: Status, Size, Life History, Geography and Habitats*. NERC Centre for Ecology
507 and Hydrology, Monks Wood, Abbots Ripton, UK.
508 Hobbs, R.J. & Norton, D.A. 2004. Ecological filters, Thresholds, and Gradients in Resistance
509 to Ecosystem Reassembly. In Temperton, V.M., Hobbs, R.J., Nuttle, T. & Halle, S.
510 (eds.). *Assembly Rules and Restoration Ecology*. pp 72-95. Island Press.
511 Hughes, F.M.R., Stroh, P.A., Adams, W.A., Kirby, K., Mountford, J.O. & Warrington, S.
512 2011. Monitoring and evaluating landscape-scale, open-ended habitat creation projects: a
513 journey rather than a destination. *Journal for Nature Conservation*
514 doi:10.1016/j.jnc.2011.02.003.

Formatted: French (France)

Formatted: German (Germany)

Jakobsson, A. & Eriksson, O. 2000. A Comparative Study of Seed Number, Seed Size, Seedling Size and Recruitment in Grassland Plants. *Oikos* 88: 494-502.Jansen, A. J. M., Grootjans, A. P. & Jalink, M. H. 2000. Hydrology of Dutch *Cirsio-Molinietum* meadows: prospects for restoration. *Applied Vegetation Science* 3:51–64.Janzen, D. H. 1982. Differential seed survival and passage rates in cows and horses, surrogate Pleistocene dispersal agents. *Oikos* 38: 150–156.

Janzen, D. H. 1984. Dispersal of small seeds by big herbivores: Foliage is the fruit. *American Naturalist* 123: 338–353. Jaroszewicz, B., Pirożnikow, E., & Sagehorn, R. 2009. Endozoochory by European bison (*Bison bonasus*) in Białowieża Primeval Forest across a management gradient. *Forest Ecology and Management* 258: 11-17.

Jongejans, E. & Telenius, A. 2001. Field experiments on seed dispersal by wind in ten umbelliferous species (Apiaceae). *Plant Ecology* 152: 67-78.

Kéry, M., Matthies, D. & Spillmann, H.H. 2000 Reduced fecundity and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. *Journal of Ecology* 88: 17–30.

Kleyer, M., Bekker, R.M., Knevel, I.C., Bakker, J.P., Thompson, K., Sonnenschein, M. et al. 2008. The LEDA Traitbase: a database of life-history traits of the Northwest European flora. *Journal of Ecology* 96: 1266–1274.

Kuiters, A.T. & Huiskes, H.P.J. 2010. Potential of endozoochorous seed dispersal by sheep in calcareous grasslands: correlations with seed traits. *Applied Vegetation Science* 13: 163-172.

Laiolo, P., Dondero, F., Ciliento, E. & Rolando, A. 2004. Consequences of pastoral abandonment for the structure and diversity of the alpine avifauna. *Journal of Applied Ecology* 44: 294-304.

Formatted: Dutch (Netherlands)

539 Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F. et al.
540 2004. The metacommunity concept: a framework for multi-scale community ecology.
541 *Ecology Letters*7: 601–613.
542 Linington, S.H. (2003). The design of seed banks, pp. 591–636 in Seed conservation, turning
543 science into practice. Eds R.D. Smith, J.B. Dickie, S.H. Linington, H.W. Pritchard and
544 R.J. Probert. pp 593-636.
545 Matus, G., Verhagen, R., Bekker, R.M. & Grootjans, A.P. 2003. Restoration of the *Cirsio*
546 *dissecti-Molinietum* in the Netherlands: can we rely on soil seed bank? *Applied*
547 *Vegetation Science* 6: 73–84.
548 Lundberg, J. & Moberg, F. 2003. Mobile link organisms and ecosystem functioning:
549 implications for ecosystem resilience and management. *Ecosystems* 6: 87-98.
550 MacArthur, R.H. & Wilson, E.O. 1967. *The theory of island biogeography*. Princeton,
551 Princeton University Press.
552 Malo, J.E. & Suárez, F. 1995. Herbivorous mammals as seed dispersers in a Mediterranean
553 dehesa. *Oecologia* 104: 246–255.
554 McCartney, M. P. & de la Hera, A. 2004. Hydrological assessment for wetland conservation
555 at Wicken Fen. *Wetland Ecological Management* 12: 189-204.
556 Meredith, T.C., & Grubb, P.J. 1993. Biological Flora of the British Isles: *Peucedanum*
557 *palustre* (L.) Moench. *Journal of Ecology* 81: 813-826.
558 Middleton, B.A. & Mason, D.H. 1992. Seed Herbivory by Nilgai, Feral Cattle, and Wild
559 Boar in the Keoladeo National Park, India. *Biotropica* 24: 538-543.
560 Middleton, B.A., Jensen, K. & van Diggelen, R. 2006. Seed dispersal in fens. *Applied*
561 *Vegetation Science* 9: 279-284.
562 Miller, M. F. 1996. Dispersal of Acacia seeds by ungulates and ostriches in an African
563 savanna. *Journal of Tropical Ecology* 12: 345-356.

Formatted: Dutch (Netherlands)

564 Moore, N. W. 1997 The Fenland Reserves In Friday, L. (Ed.) *Wicken Fen: the making of a*
565 *wetland nature reserve,* pp. 3-8. Harley Books, Colchester, UK.
566 Mouissie, A.M. 2004. *Seed dispersal by large herbivores: implications for the restoration of*
567 *plant biodiversity*. Ph.D. thesis. University of Groningen.
568 Mouissie, A.M., Vos, P., Verhagen, H.M.C. & Bakker, J.P. 2005. Endozoochory by free
569 ranging, large herbivores: ecological correlates and perspectives for restoration. *Basic*
570 *and Applied Ecology* 6: 547– 558.
571 Murphy, M. R., Kennedy, P. M. & Welch, J. M. 1989. Passage and rumination of inert
572 particles varying in size and specific gravity as determined from analysis of faecal
573 appearance using multicompartmental models. *British Journal of Nutrition* 62: 481-492.
574 Naujeck, A. & Hill, J. 2005. Influence of sward height on diet selection by horses. *Applied*
575 *Animal Behavioural Science* 90: 49 - 63.
576 Ozinga, W.A., Bekker, R.M., Schaminée, J.H.J. & van Groenendael, J.M. (2004). Dispersal
577 potential in plant communities depends on environmental conditions. *Journal of Ecology*
578 92: 767–777.
579 Ozinga, W.A., Römermann, C., Bekker, R.M., Prinzing, A., Tamis, W.L.M., Schaminée,
580 J.H.J., Hennekens, S.M., Thompson, .K., Poschlod, P., Kleyer, M., Bakker, J.P. & van
581 Groenendael, J.M. 2009. Dispersal failure contributes to plant losses in NW Europe.
582 *Ecology Letters* 12: 66–74.
583 Pakeman, R. J. 2001. Plant migration rates and seed dispersal mechanisms. *Journal of*
584 *Biogeography* 28: 795-800.
585 Pakeman, R. J., Digneffe, G. & Small, J. L. 2002. Ecological correlates of endozoochory by
586 herbivores. *Functional Ecology* 16: 296–304.

587 Peco, B., de Pablos, I., Traba, J. & Levassor, C. (2005). The effect of grazing abandonment
588 on species composition and functional traits: the case of Dehesa grasslands. *Basic and*
589 *Applied Ecology* 6: 175–183.
590 Poschlod, P., Bakker, J.P. & Kahmen, S. 2005. Changing land use and its impact on
591 biodiversity. *Basic and Applied Ecology* 6: 93-98.
592 Prentice, H. C. & Andersson, S. 1997. Genetic variation and population size in the rare
593 dioecious plant, *Silene diclinis* (Caryophyllaceae). In: Tew, T. E., Crawford, T. J.,
594 Spencer, J. W., Stevens, D. P., Usher, M. B. and Warren, J. (eds) *The Role of Genetics*
595 *in Conserving Small Populations*, pp. 65–72. JNCC, Peterborough, UK.
596 Pywell, R.F., Bullock, J.M., Hopkins, A., Walker, K.J., Sparks, T.H., Burke, M.J.W. & Peel,
597 S. 2002. Restoration of species-rich grassland on arable land: assessing the limiting
598 processes using a multi-site experiment. *Journal of Applied Ecology* 39: 294-309.
599 Rodwell, J. S. 1991. *British Plant Communities, vol. 2, Mires and Heaths*, Cambridge
600 University Press, UK.
601 Simao Neto, M., Jones, R. M. & Ratcliff, D. 1987. Recovery of pasture seed ingested by
602 ruminants. 1. Seed of six tropical pasture species fed to cattle sheep and goats.
603 *Australian Journal of Experimental Agriculture* 27: 239–246.
604 Smart, S. 2000. *Modular Analysis of Vegetation Information System (MAVIS).* Centre for
605 Ecology and Hydrology, Lancaster, UK.Soons, M.B., Messelink, J.H., Jongejans, E.
606 & Heil, G.W. 2005. Habitat fragmentation reduces grassland connectivity for both
607 short-distance and long-distance wind-dispersed forbs. *Journal of Ecology* 93: 1214–
608 1225.
609 Stace, C.A. 2010. *New Flora of the British Isles*. Third Edition. Cambridge University Press,
610 UK.

611 Tackenberg, O., Poschlod, P. & Bonn, S. 2003. Assessment of wind dispersal potential in
612 plant species. *Ecological Monographs* 73:191-205.
613 Ter Heerdt, G.N.J., Verweij, G.L., Bekker, R.M. & Bakker, J.P. 1996. An improved method
614 for seed bank analysis: seedling emergence after removing the soil by sieving.
615 *Functional Ecology* 10, 144–151.
616 Traba, J., Levassor, C. & Peco, B. 2003. Restoration of species richness in abandoned
617 Mediterranean grasslands: seeds in cattle dung. *Restoration Ecology* 11: 378–384.
618 van Braeckel, A. & van Looy, K. 2002. *Monitoring of Nature Management along the Meuse. Preliminary results from Kerkeweerd*. Report Institute of Nature Conservation,
620 Brussels.
621 Thompson, K., Bakker, J. P. & Bekker, R. M. 1997. The soil seed banks of North West
622 Europe Methodology, Density and Longevity. Cambridge University Press, Cambridge,
623 UK..
624 Vaughan, I.M. 1978. *Lathyrus palustris* (L.) var *pilosus* (Cham.) Ledeb in V.C.44. *B.S.B.I. News* 20: 14.
626 Vellend, M., Myers, J. A., Gardescu, S. & Marks, P. L. 2003. Dispersal of Trillium seeds by
627 deer: implications for long-distance migration of forest herbs. *Ecology* 84: 1067-1072.
628 Welch, D. 1985. Studies in the grazing of heather moorland in north-east Scotland. IV. Seed
629 dispersal and plant establishment in dung. *Journal of Applied Ecology* 22: 461–472.
630 Wigbels, V. 2000. *Oostvaardersplassen: New Nature below Sea Level*. MMI Staatsbosbeheer
631 Flevoland-Overijssel.
632
633

Formatted: German (Germany)
Formatted: Dutch (Netherlands)
Formatted: Dutch (Netherlands)